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Dolichol and dolichyl phosphate in human tissues

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Summary. The content of dolichol and dolichyl phosphate in various human organs was analysed using autopsy samples. The reliability of these measurements was demonstrated by comparison with values for fresh biopsy material. Dolichol was present in all tissues investigated and the content was highest in the adrenal gland, pancreas, pituitary gland, testis and thyroid gland, ranging between 1.5 and 7.1 mg/g tissue. Dolichyl-P was detected in the various organs in highly variable amounts, ranging between 1 and 9% of the total dolichol content. While the main pattern of isoprene composition for dolichol and dolichyl-P was similar in individual organs, some variation was observed between tissues. Dolichol represents the largest lipid component in the pituitary gland, exceeding the total phospholipid content. The high concentrations of dolichol and dolichyl-P in human organs indicate that these lipids may play important roles in physiological and pathological cellular functions.

Keywords: dolichol, dolichyl phosphate, human tissues, isoprenoid pattern, pituitary gland

During the last decade a number of investigations designed to identify and quantify dolichol in eukaryotic cells and membranes have been performed. Dolichol is widely distributed and varies greatly in amount between different species, tissues and intracellular structures (Dallner & Hemming 1981; Hemming 1983). Tissues of rat, chicken, pig, rabbit and bovine origin were found to contain 10–200 μg total dolichol/g wet weight, including that portion of the dolichol which is esterified with a fatty acid. In an early investigation employing column chromatography and gravimetry the authors concluded that most human tissues also contain large quantities of this lipid (Rupar & Carroll 1978), although at that time no reliable method for quantification of dolichyl-P was available. The dolichol and dolichyl-P contents of several human organs were quantified some years later, but the reliability of the assay procedures was still unsatisfactory (Eggens *et al.* 1983).

It has been established that dolichyl phosphate is an essential intermediate in the synthesis of *N*-glycosidically-linked oligosaccharide chains and in several tissues the level of this substance is rate-limiting for the process of glycosylation (Mills & Adamany 1978; Carson & Lennarz 1979; Potter *et al.* 1981; Eggens *et al.* 1984). On the other hand, the function of non-phosphorylated dolichol, present in part as a free alcohol and partially as a fatty acid ester and representing 90% or more of the total dolichol content, has not yet been established. In attempts to

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understand the function of dolichol, the study of its distribution in various human tissues and cell fractions is important.

In this study the distribution of dolichol and dolichyl phosphate in human autopsy material was investigated. The validity of the assay procedure was confirmed by comparison with corresponding values for fresh biopsy material. The results demonstrated that polyisoprenoids can be determined in autopsy samples. A preliminary report on some of the present findings has already been published (Tollbom 1985).

Materials and methods

Autopsy material taken within 2 days after death from patients ranging in age from 68 to 74 years was used. Only tissues which appeared histologically normal were processed. Where indicated, fresh biopsy samples, taken for diagnostic purposes or from specimens removed surgically, were frozen immediately after removal and later used for extraction.

Tissues were homogenized with an Ultra-Turrax blender and supplemented with known amounts of dolichol-15 and -23, and dolichyl-15 and -23-phosphate as internal standards, as described previously (Eggens *et al.* 1983). The lipids were extracted for 4 h at 40°C with chloroform:methanol:water (CMW) 1:1:0.3 using a magnetic stirrer. By comparison with values obtained by extensive extraction in a Soxhlet apparatus, it could be shown that dolichol and dolichyl-P were extracted completely. Partition was achieved by adjusting the CMW mixture to 3:2:1. The chloroform phase was subsequently removed and washed four times with theoretical 'upper phase' by thorough mixing and centrifuging. The solvent was evaporated under N_2 and the extract dissolved in CMW, 1:1:0.3. This latter solvent mixture was used for equilibration of a DEAE-Sephadex column (acetate form, 0.7×13 cm). Dolichol and dolichyl esters were eluted from this column with 20 ml of CMW, 1:1:0.3, and dolichyl phosphate was then released using 200 mM ammonium acetate in CMW, 1:1:0.3. Controls with these three types of polyprenol demonstrated their separation and elution to be complete.

The dolichol-containing mixture was brought to 3:2:1 with CMW and washed twice by thorough mixing with upper phase and centrifuging. The solvent was evaporated and the residue subjected to alkaline hydrolysis using 1 ml 60% KOH and 2 ml 0.25% pyrogallol in methanol in order to hydrolyze triglycerides, cholesterol and dolichol ester. The sample was maintained at 80°C for 45 min, followed by the addition of 3 ml chloroform. The chloroform phase thus obtained was washed three times with upper phase, evaporated, and the lipid dissolved in 5 ml methanol. This mixture was then transferred to Sep Pac C18 (Waters), washed with 20 ml methanol, eluated with hexane, dried and dissolved in hexane. The eluate contained not only the original dolichol fraction, but also the dolichol esters converted to free dolichols by alkaline hydrolysis. The fraction referred to subsequently as dolichol thus also contains the original dolichol ester fraction.

HPLC analyses were performed with a Hewlett-Packard Hypersil ODS 3 μm reversed phase column. A convex gradient was used (nr 5, Waters 660 Solvent program), from the initial 2-propanol:methanol:H_2O, 40:60:5, in pump system A to 40% hexane:2-propanol, 70:30, in pump system B at a flow rate of 1 ml/min and a program time of 40 min. The absorbance of the eluate at 210 nm was monitored.

The dolichyl-P fraction eluted from the DEAE-Sephadex column was adjusted with CMW to 3:2:1 and washed five times with upper phase in order to remove ammonium acetate. After evaporation of the solvent, 5 ml chloroform:methanol, 3:2, and concentrated HCl to a final concentration of 0.3 M was added. The acid hydrolysis was performed in two steps: the sample was maintained at 20°C for 45 min and then incubated at 65°C for 45 min. Initial mild acid hydrolysis removes monosaccharides from

dolichyl phosphates and the stronger acid hydrolysis removes oligosaccharide phosphate from dolichyl pyrophosphate. Dolichyl-P was completely resistant to acid hydrolysis as demonstrated using labelled substances and reisolation. The mixture was subsequently evaporated, supplemented with 2 ml methanol, 2 ml H_2O and 1 ml 60% KOH and alkaline hydrolysis performed at 85°C for 45 min. The hydrolyzed sample was adjusted to a CMW ratio of 3:2:1 and washed with upper phase four times. After evaporation, the lipids were dissolved in 100 μl HPLC mixture A containing 20 mM phosphoric acid. HPLC analyses were performed as described for dolichol, but with the inclusion of 20 mM phosphoric acid in both solvent mixtures A and B.

Protein was measured by the Biuret method using bovine serum albumin as standard (Gornall *et al.* 1949). Phospholipids were separated by thin layer chromatography and individual species quantified by phosphate determination (Valtersson & Dallner 1982).

Table 1. Dolichol content in human organs

Tissue	Dolichol (μg/g wet weight)
Adrenal	1598±168
Aorta	108±16
Brain	279±36
Colon	82±8
Fatty tissue	170±11
Heart	185±22
Kidney	192±25
Liver	452±38
Lung	247±12
Muscle	393±40
Ovary	128±18
Pancreas	1440±121
Pituitary gland	7168±592
Placenta	15±3
Prostate	79±15
Small intestine	153±13
Spleen	114±13
Testis	1542±140
Thyroid gland	1960±198
Uterus	481±58
Stomach	182±26

The values represent the mean of 6–11 measurements ± SE.

Results

The human material used in this investigation consisted of autopsy samples stored at −20°C until use. Table 1 shows the dolichol content of various human organs. The highest dolichol content, on a weight basis, is found in the pituitary gland (7 mg/g), whereas the adrenals, testis, thyroid and pancreas all have dolichol contents ranging between 1 and 2 mg/g. Most other organs also contain high concentrations of dolichol, ranging from 100 to 500 μg/g. Most human tissues thus have far higher dolichol contents than do those of laboratory animals. Some human tissues, however, do have a very low (placenta) or low (colon, prostate) dolichol content when assessed on a wet weight basis, but even in these cases, the actual dolichol content may be much higher when calculated on a specific cell or tissue basis. It is probable that in an organ with a heterogeneous cellular composition, the dolichol contents of different types of cells differ greatly and some cells may have a dolichol content as high or even higher than that of pituitary or thyroid cells.

The level of dolichyl-P in different human tissues varies greatly and is in all cases much lower than that of the free alcohol (Table 2). In some tissues there is less than 10 μg/g of this lipid while in others its concentration is much higher. This variation remains even when the level of dolichyl-P is expressed as a percentage of the total dolichol, for 10-fold differences between certain tissues could be observed.

Well characterized enzyme systems are known to interconvert dolichol and dolichyl-P, eg. dolichol kinase and dolichol mono- and pyrophosphatase (Allen *et al.* 1978; Burton *et al.* 1979; Rip *et al.* 1981; Appelkvist *et al.*

Table 2. Dolichyl-P content in human organs

	Dolichyl-P (μg per g wet weight)		
	without acid hydrolysis	with acid hydrolysis	% of total dolichol*
Adrenal		51.1±4.1	3.1
Aorta		10.2±1.0	8.6
Colon		3.1±0.5	3.6
Heart	6.5±1.5	13.1±1.0	6.6
Kidney		12.7±1.5	6.2
Liver	6.1±1.1	18.8±2.3	4.0
Lung	9.7±1.2	13.3±1.0	5.1
Muscle	6.6±1.3	16.4±1.6	4.0
Pancreas	9.4±1.5	30.9±3.2	2.1
Pituitary gland		283.1±22.2	3.8
Prostate		8.2±1.2	9.4
Small intestine	4.8±0.9	11.9±1.1	7.2
Spleen	1.8±0.9	3.3±0.6	2.8
Testis	68.3±6.1	72.7±5.5	4.5
Thyroid gland	9.4±0.8	13.8±1.2	0.7
Stomach		2.6±0.5	1.4

The values represent the mean of 5–8 measurements ± SE.
* Total dolichol is the sum of the free alcohol content given in Table 1 and the amount of dolichyl-P obtained after acid hydrolysis.

1981; Scher & Waechter 1984), and there is experimental evidence that these enzymes are functional *in vivo* (Rossignol *et al.* 1983). The variations in levels of dolichyl-P in different tissues shown here imply that the free alcohol and the phosphorylated form are not necessarily biosynthetically interrelated as was suggested for rat liver (Ekström *et al.* 1984). Table 2 also shows dolichyl-P content before acid hydrolysis, ie. lipid intermediates not covalently linked to mono- or oligosaccharides (Keller *et al.* 1981; Warren *et al.* 1975). In most tissues a relatively large proportion of the total dolichyl phosphate has sugars attached, indicating participation in glycoprotein synthesis.

The tissue distributions of individual dolichols and dolichyl phosphates are shown in Table 3. The largest dolichol component contains 19 isoprene residues and constitutes 41–55% of the total. Most of the rest consists of D18 and D20. D18 can be equal to, larger, or smaller in amount than the dolichol with 20 isoprene residues. Table 3 also shows the dolichyl-P composition of those tissues for which the dolichol distribution is given. Dolichyl-18-P and -20-P are present in large amounts in all tissues, but dolichyl-19-P is most abundant. As with the free alcohols, the relative amounts of dolichyl-18-P and -20-P vary, but their distribution patterns differ somewhat from those of the corresponding free alcohols.

Use of autopsy material is only valid if the substance to be analysed is stable postmortem until the tissue is processed. To check

Table 3. Compositions of dolichol and dolichyl-P in human tissues

Tissue	Composition (% of total)				
	D17	D18	D19	D20	D21
Dolichol					
Adrenal	3	26	52	19	0
Aorta	2	32	55	10	1
Fatty tissue	3	22	53	20	2
Liver	4	12	49	27	8
Muscle	3	34	46	15	2
Testis	5	26	41	24	4
Thyroid gland	3	17	44	32	4
Stomach	1	22	53	23	1
Dolichyl-P					
Adrenal	3	27	49	17	4
Aorta	3	20	47	23	7
Fatty tissue	2	20	56	21	1
Liver	1	28	42	26	3
Muscle	2	30	50	16	2
Testis	4	17	43	32	4
Thyroid gland	2	21	52	24	1
Stomach	2	30	48	18	2

These values are the means of five experiments.

Table 4. Comparison of the contents of dolichol and dolichyl-P in autopsy and fresh human biopsy material

Tissue	Autopsy material (μg/g wet weight)	Fresh biopsy or surgical sample (μg/g wet weight)
Liver		
dolichol	452	426
dolichyl-P	18.8	17.9
Muscle		
dolichol	393	413
dolichyl-P	16.4	17.8
Stomach		
dolichol	182	188
dolichyl-P	2.6	3.3

These values are the means of six to ten measurements on autopsy material and of three measurements in the case of biopsy material.

that postmortem degradation was not yielding incorrectly low values, we compared autopsy and surgical biopsy samples of liver, muscle and stomach (Table 4). The levels of total dolichol (free-alcohol + esterified form) and dolichyl-P are comparable in both types of material. The determination of both types of polyisoprenoid compounds can thus be performed accurately on autopsy samples and the results obtained reflect the *in vivo* situation.

The pituitary gland is interesting for its extremely high dolichol content. This organ contains phosphatidylcholine and phosphatidylethanolamine as its two main phospholipids and some phosphatidylserine, phosphatidylinositol and sphingomyelin as well (Table 5). It also contains cholesterol, (circa 30%), and triglycerides (circa 5% of the total phospholipid content) (not shown in Tables). Similar results were reported by Singh & Carroll 1969. Dolichol is thus the largest lipid component of this organ.

The high dolichol content of the pituitary gland could reflect the presence of this lipid in membranes, in non-membraneous form within organelles or in the soluble cytosolic fraction. Subfractions of pituitary gland homogenates were prepared (Table 6) which, it is assumed, were enriched with nuclei, heavy and light mitochondria and microsomes, depending on the centrifugal force employed. The 18 000 ct/min fraction which should contain lysosomes or secretory vesicles, has the highest dolichol content, but all other membrane fractions also contain this lipid. Fresh human pituitary gland was not available for detailed electron microscopy and marker enzymes studies and these assumptions thus await experimental confirmation. However, dolichol was clearly distributed unevenly between the different subfractions.

Discussion

The dolichol and dolichyl-P contents of various human organs were analysed in histologically normal autopsy material. Methods

Table 5. Lipid content of human pituitary gland

Lipid	Amount (mg/g wet weight)	Amount (% of total)
Total phospholipid	6.27±1.04	100.0
Phosphatidylcholine	2.66±0.55	42.5
Phosphatidylethanolamine	0.99±0.13	15.8
Lysophosphatidylethanolamine	0.63±0.04	10.1
Phosphatidylserine	0.31±0.02	5.0
Phosphatidylinositol	0.38±0.03	6.1
Sphingomyelin	0.82±0.09	13.0
Dolichol	7.168	
Dolichyl-P	0.283	

These values represent the means of six measurements ± SE.

Table 6. Distribution of dolichol in subfractions of human pituitary gland

Fraction*	Dolichol (mg/g wet weight)		Dolichol (% of total)
Total	7.29	7.06	100
4000 ct/min			12.1±6.0
6500 ct/min			15.5±2.1
18 000 ct/min			49.7±5.2
40 000 ct/min			19.1±1.4
Supernatant			2.6±0.5

These values are the means of five measurements ± SE.

* The fractions represent pellets obtained after serial centrifugation (Beckman, 50 TI rotor) of the homogenate at 4000 ct/min (10 min), 6500 ct/min (10 min), 18 000 ct/min (10 min) and 40 000 ct/min (60 min). The supernatant was obtained by centrifuging the supernatant fraction from the 40 000 ct/min pellet at 40 000 ct/min for an additional 2 h.

have become available only recently for the exact quantitation of these lipids, hence, values obtained here differ slightly from those reported in earlier (Rupar & Carroll 1978; Eggens *et al.* 1983).

The results obtained appear to reflect accurately *in vivo* conditions. Autopsy material is inadequate for many biochemical studies because of postmortem changes. However, both dolichol and dolichyl-P were

stable in the autopsy material used here. It is not known how dolichol is degraded and the main route for removal of these lipids may be excretion rather than breakdown (Connelly & Keller 1984). Dolichol mono- and pyrophosphatases are active in rat liver and they may be present also in human organs. However, the fact that dolichyl-P is not hydrolyzed in human autopsy tissue is not necessarily surprising for the hydrolytic enzymes could be blocked or localized in a different compartment from that of the lipid substrate.

A large part of dolichol in animals is known to be present as fatty acid esters, but the extent of esterfication was not determined here. In this study, dolichol was analysed after complete alkaline hydrolysis of all dolichol esters. In experiments using rat tissues we found extensive postmortem hydrolysis of dolichol esters and the use of autopsy material for such analyses is thus questionable. The extent of esterification in fresh human biopsies requires investigation.

In comparison with corresponding organs of the rat, chicken, rabbit and pig, human tissues contain unusually high levels of dolichol and dolichyl-P (Dallner & Hemming 1981). While dolichyl-P clearly plays a role as an obligatory intermediate in the biosynthesis of *N*-glycosidically-linked oligosaccharide chains, the function of the free alcohol is presently unknown. Dolichol may be partly localized within cytoplasmic granules and vesicles or in the supernatant, but much of this lipid also appears to be present in membranes. Dolichol destabilizes membrane phospholipid structures and increases fatty acid fluidity and permeabilty, thereby altering membrane properties (Lai & Schutzbach 1984; Vigo *et al.* 1984; Valtersson *et al.* 1985). The remarkably high dolichol content in endocrine tissue is interesting, but it is not clear whether this finding is related to hormone production or secretion.

The dolichol content of human brain (Pullarkat & Reha 1982) and rat liver (Ganning *et al.* 1984) increases with age; thus, physiological factors may regulate the distribution of this lipid. This content also decreases dramatically during cirrhosis and primary liver cancer in humans and other pathological processes may also influence its distribution (Eggens *et al.* 1983; 1984; Ng Ying Kin & Wolfe 1983; Keller *et al.* 1984).

The differences in the distributions of the free alcohol and the phosphorylated form raise the question of whether dolichol is simply the precursor of the phosphorylated lipid in human tissue or whether it is present in cellular structures for other reasons. These differences would be in accordance with the previous suggestion that separate biosynthetic routes for dolichol and dolichyl-P may exist in rat liver (Ekström *et al.* 1984).

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